In vivo imaging of dopamine and serotonin release

Udo de Haes, Joanna Irene

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
CHAPTER 5

ASSESSMENT OF METHYLPHENIDATE-
INDUCED CHANGES IN BINDING OF
CONTINUOUSLY INFUSED
$[^{11}\text{C}]$-RACLOPRIDE IN HEALTHY HUMAN
SUBJECTS: CORRELATION WITH
SUBJECTIVE EFFECTS

J. I. Udo de Haes$^1$, R. Kortekaas$^{2,3}$, A. Van Waarde$^4$, R.P. Maguire$^{2,5}$, J.Pruim$^6$,
J. A. den Boer$^1$

$^1$Department of Biological Psychiatry, University Medical Center Groningen, $^2$Department
of Neurology, University Medical Center Groningen, $^3$Department of Anatomy and
Embryology, University Medical Center Groningen, $^4$PET center, University Medical
Center Groningen, $^5$Pfizer Global Research and Development, USA

Psychopharmacology, in press
Abstract

The dopaminergic system has been implicated in the pathogenesis and treatment of a variety of neuropsychiatric disorders. It has been shown that information on endogenous dopamine (DA) release can be obtained noninvasively, by combining positron emission tomography (PET) with a dopaminergic challenge. This approach is based on the assumption that an injected radiolabeled ligand competes with the neurotransmitter for the same receptor. Increases in DA release will therefore result in a decreased binding of the radioligand. We investigated the effect of the DA reuptake blocker methylphenidate (MP) (0.25 mg/kg i.v) on the binding of the D2 receptor ligand [11C]-raclopride (RAC) in 6 healthy volunteers. RAC was administered as a bolus followed by constant infusion and subjective effects were assessed using verbal rating scales. Control scans without MP administration, showed that the mean RAC binding reached stable values approximately 30 minutes after start of the infusion. MP administration induced a 24% decrease in RAC binding in the total striatum. Significant correlations were found between the MP-induced change in euphoria and percent change in binding potential (ΔBP) in the dorsal striatum and between baseline anxiety and ΔBP in the dorsal and middle striatum. We also found a significant negative correlation between baseline BP in the dorsal striatum and change in euphoria. Our results comply with previous findings, indicating the feasibility of the bolus infusion design combined with a relatively low MP dose to study dopaminergic (dys)function.
Introduction

The dopaminergic system has been implicated in the pathogenesis and treatment of a variety of neurological and psychiatric disorders such as schizophrenia, Parkinson’s disease, depression and addiction (Kapur and Mamo 2003; Leenders 2002; Naranjo et al. 2001; Volkow et al. 2002a). The involvement of dopamine (DA) in these disorders may be related to its function in reward processing (Kapur 2003; Kunig et al. 2000; Schmidt et al. 2001). However, the exact role of DA in these processes is not completely known yet. In order to study the (dys)function of the dopaminergic system, it is important to quantify changes in DA levels in the living brain.

Over the last decade, it has been shown that information on endogenous DA release can be obtained noninvasively, by using positron emission tomography (PET) or single-photon emission computed tomography (SPECT). This approach is based on the assumption that an injected radiolabeled ligand competes with the endogenous neurotransmitter for the same receptor. Increases in DA release will result in a decreased binding of the radioligand and vice versa (Dewey et al. 1993; Laruelle 2000). The exact mechanism for these changes in receptor availability is not known. The changes may be a result of true competition between DA and the injected ligand, but may also be due to DA-induced internalization of the receptor (Sun et al. 2003). Nevertheless, it is generally assumed that the changes in radioligand binding can be used as a measure for the alterations in neurotransmitter release (Laruelle 2000). Using this method, correlations between DA function and behavioral or physiological scores have been found, providing information on the possible role of this neurotransmitter in pathological or physiological processes (Drevets et al. 2001; Laruelle et al. 1995; Leyton et al. 2002; Martinez et al. 2003; Pruessner et al. 2004; Volkow et al. 1994, 1999a, 2003).

Previous studies that investigated changes in DA levels, have primarily assessed changes in striatal binding, using D2 receptor antagonists such as [11C]-raclopride (RAC) or [123I]-IBZM. In most studies a paired bolus protocol was used, with two separate scans: a dopaminergic challenge is
administered during one of the scans while the other scan serves as a control. Alternatively, the ligand may be administered as a bolus followed by a constant infusion, leading to sustained equilibrium of radioligand levels in blood and brain (Carson 2000). A challenge can then be administered during the equilibrium period, enabling measurement of baseline and drug-induced changes in ligand binding in the same experiment. During equilibrium, there is no net transfer of the radioligand across the blood-brain barrier, thereby minimizing possible effects of changes in drug-induced blood flow on ligand binding (Laruelle 2000). This aspect is an important advantage of the bolus with infusion paradigm, as compared to the double bolus protocols.

Previous studies using RAC as a bolus followed by infusion have used amphetamine (AMPH) to assess pharmacologically-induced changes in dopaminergic transmission (Breier et al. 1997; Carson et al. 1997; Martinez et al. 2003; Tsukada et al. 2002). Since AMPH is not a registered drug in the Netherlands, we aimed to investigate the effect of the DA reuptake blocker methylphenidate (MP). Microdialysis studies have shown that both compounds induce large increases in DA levels (Breier et al. 1997; Hernandez et al. 1987; Hurd and Ungerstedt 1989; Kuczenski and Segal 1997) which resulted in 15-25% reductions in RAC binding in human subjects (Breier et al. 1997; Drevets et al. 2001; Martinez et al. 2003; Piccini et al. 2003; Volkow et al. 1994). The effect of MP on RAC binding has only been investigated using the double bolus method. Using this method, Volkow et al. (1999a) studied the effect of a wide range of MP doses and found significant changes in specific binding after intravenous doses of 0.25 mg/kg and 0.5 mg/kg. The effect of MP on RAC binding has not yet been investigated using a bolus with constant infusion protocol. In our study we used this protocol to investigate the effect of MP in healthy volunteers. In future studies we intend to use this method to study the dopaminergic system in psychiatric patients. We used a MP dose of 0.25 mg/kg, i.v., since in previous studies comparable doses were used in psychiatric patients (Janowsky and Davis 1976; Joyce et al. 1986). In order to check the equilibrium state of the ligand, we also investigated the protocol without MP administration. We assessed the subjective state of the subject before and after MP administration, since previous studies reported correlations
between subjective effects and changes in ligand binding (Drevets et al. 2001; Laruelle et al. 1995; Leyton et al. 2002; Martinez et al. 2003; Volkow et al. 1994, 1999a). Finally, studies using AMPH have found a larger effect on ligand binding in more ventral compared to dorsal regions of the striatum. Therefore, we studied the effect of MP in different striatal regions.

The aim of our study was to investigate if the results from the combined use of a relatively low dose of MP with a RAC constant infusion protocol comply with results from previous studies that used different dopaminergic challenges or double bolus injections.

Materials and Methods

Subjects
Six healthy volunteers participated in the study (5 male, 1 female, mean age 24 years, range 18-32 years). All subjects gave written informed consent after written and oral explanation of the study. Suitability to participate in the study was determined by an independent physician after a medical examination including an ECG and routine blood hematology and biochemistry tests. Exclusion criteria were current or past psychiatric, neurological or other diseases that could interfere with the study, dependence on any substance other than nicotine or caffeine and exposure to psychoactive drugs during the past 3 months. The study was approved by the Medical Ethics Committee of the University Medical Center Groningen (UMCG).

[^11C]-raclopride synthesis
The tracer[^11C]-raclopride was prepared from[^11C]-methyl iodide as previously described by Ehrin et al. (1987). It was dissolved in a volume of 20 mL sterile saline, and taken to the subject in the PET scanner. The specific radioactivity was >10,000 GBq/mmol at the time of injection.
PET scan protocol

The subjects were instructed to refrain from alcohol and caffeine containing products during 24 hours prior to each scan. Before the start of the PET scan an intravenous catheter was inserted in each arm for blood sampling and MP injection and for ligand administration. The volunteers were positioned in the camera and their heads were fixed using a head restraint. The subjects were scanned in 3D acquisition mode using a Siemens ECAT Exact HR+ camera, giving 63 slices with a center to center distance of 2.425 mm. During the scans, RAC was administered as a fast bolus (1 minute) followed by a constant infusion over 90 minutes with a bolus to infusion rate ratio \((K_{bol})\) of 100 minutes. The mean \((\pm SD)\) activity at the start of the experiment was 220 \((\pm 99)\) MBq. Thirty-six consecutive frames were acquired with a duration of 2.5 minutes. Each subject was scanned twice. One of the scans served as a control scan to check the equilibrium state of the ligand. During the other scan MP 0.25 mg/kg was injected intravenously over 1 minute, at 40 minutes after start of the RAC infusion. The two scans were performed in random order separated by 2 -7 months. During both scans plasma samples were taken for metabolite analysis at 5, 30, 60 and 90 minutes after the start of the RAC infusion. During the MP scan, subjective and behavioral effects were evaluated using a verbal analog rating scale, at 10 minutes before and 10 minutes after MP injection. The subjects were asked to respond to the following descriptors, using a whole number between 0 (no effects) and 10 (maximal effects): Euphoria, anxiety, happiness, sexual desire, desire for MP, alertness, annoyance, distrust, loss of control, restlessness, depression (Volkow et al. 1999a,b; Wang et al. 1997). Blood pressure, heart rate and ECG were continuously monitored during the MP scans.

Metabolite analysis

Plasma samples from only five subjects were analyzed due to sampling problems in one of the volunteers. After centrifugation with acetonitrile, the liquid phase of the samples was injected into the HPLC system, which contained a 300 x 7.9 mm \(\mu\)Bondapak C18 semi-preparative column. The column was eluted with a mixture of acetonitrile / water / 85% phosphoric acid (500:500:1) at a flow rate of 2 ml/min. Fractions of the eluate were collected at 0.5 min intervals and radioactivity in the fractions was
determined using a gamma counter. The relative contributions of parent and metabolites were calculated.

**Image analysis**

Attenuation correction was performed by drawing ellipses on the brain images, assuming uniform attenuation. Statistical Parametric Mapping (SPM) 99 was used for spatial normalization of the RAC image. A summation of the first 10 RAC frames was normalized to the SPM $[^{15}\text{O}]$-H$_2$O template, giving 68 horizontal planes. The normalization parameters were thereafter used to normalize the other frames of the RAC scan. A predetermined standard region of interest (ROI) template localized in stereotactic MNI space was applied to determine the ROIs including left and right dorsal, middle and ventral caudate nucleus, dorsal and ventral putamen, cerebellum and occipital cortex. For further analyses we defined the following three striatal subregions: dorsal (mean of six horizontal slices containing the dorsal caudate and putamen), middle (mean of 4 horizontal slices containing the middle caudate and ventral putamen) and ventral (mean of 3 horizontal slices containing the ventral caudate, including the nucleus accumbens). An activity threshold of 30% was used to extract stable peak values from the ROIs (Rottenberg et al. 1991).

Time activity curves from striatal and occipital regions were obtained. Estimates of the mean BP were calculated with $\frac{(C_t-C_{ref})}{C_{ref}}$ (Ito et al. 1998), where $C_t$ is the mean activity over a time interval (T) in the region of interest and $C_{ref}$ is the mean activity over T in a reference region. Intervals were $T_1$ (32.5-40 minutes) and $T_2$ (65-80 minutes). During $T_1$, RAC binding was relatively stable, as assessed by visual inspection of the time-activity curves. $T_2$ was chosen as a compromise between effect size and signal to noise ratio. In our study we selected the occipital cortex as reference region since the activity in the cerebellum was relatively noisy, probably due to low injected activity. The density of $D_2$ receptors is negligible in the occipital region compared to the striatum (Camps et al. 1989; Lidow et al. 1989), and has been used before as a reference region for $D_2$ binding (Booij et al. 1997; Kegeles et al. 1999; Laruelle et al. 1995; Laruelle et al. 1997). The baseline subjective scores and the scores after MP minus baseline score were used as outcome measures for the behavioral effects.
Statistical analysis

Differences between $BP(T_1)$ and $BP(T_2)$ were calculated for the control and MP scans, using a paired t-test. Percent change in $BP$ ($\Delta BP$) was calculated as follows: $(BP(T_2) - BP(T_1) / BP(T_1)) \times 100$. $\Delta BP$ was assessed for the control and MP scans for the total striatum and the striatal subregions (dorsal, middle and central) and differences in $\Delta BP$ between the control and MP condition were calculated using paired t-tests. The effect of MP on the subjective scores was assessed using paired t-test. Pearson product moment correlations were calculated between the behavioral changes and $\Delta BP$ (difference between $\Delta BP$ in the control and MP condition) in the striatal subregions. Based on previous studies we investigated the following correlations: $\Delta BP$ with MP-induced change in euphoria, anxiety at baseline with $\Delta BP$ and baseline $BP$ (mean of control and MP scans) with change in euphoria (Drevets et al. 2001; Laruelle et al. 1995; Martinez et al. 2003; Volkow et al. 1994, 1999a,b, 2002b). The effect of MP on blood pressure and heart rate was tested with a paired t-test. The four values measured during an interval of 30 minutes before start of the PET scan were compared to the six values obtained between four to eighteen minutes after MP administration which was the time period when peak effects occurred.

Drugs

Methylphenidate was obtained from Fagron farma BV, the Netherlands and infusions were prepared and provided by the pharmacy of the UMCG.

Results

Metabolite analysis

As in previous studies, raclopride metabolism was not significantly altered by MP (Figure 1) (Drevets et al. 2001; Martinez et al. 2003; Volkow et al. 1994).
Cardiovascular measurements

MP significantly increased heart rate (p < 0.001) and systolic (p < 0.001) and diastolic blood pressure (p < 0.001). Increases ranged from 40-70% (heart rate), 20-40% (systolic blood pressure) and 20-30% (diastolic blood pressure). The effects on heart rate and blood pressure are in agreement with results from previous studies (Volkow et al. 2003). No significant changes were observed in the ECG, apart from the increases in heart rate.

PET measurements

Mean time-activity curves for the control scans and MP scans (total binding and ratio to the occipital cortex) are shown in fig 2. In the control condition, the mean RAC $BP$ reached relatively stable values approximately 30
120 minutes after the start of the infusion. MP administration induced a decrease in RAC binding in the striatum, whereas binding in the occipital cortex was not affected. A paired t-test showed a significant difference between striatal BP before \((BP(T_1))\) and after \((BP(T_2))\) MP administration \((p< 0.001)\) whereas in the control condition no difference was found between \(BP(T_1)\) and \(BP(T_2)\) \((p = 0.470)\). Mean \(\Delta BP\) in the total striatum after MP injection was -24% (range -14 to -40%), whereas the mean \(\Delta BP\) in the control condition was 0% (range +33 to -21%).

Since not all individual time activity curves in the control situation were in equilibrium, we used the difference in \(\Delta BP\) between the MP and control scans, for further analyses. The mean difference in \(\Delta BP\) between the MP and control condition was -24% (range 0 to -48%). The difference in \(\Delta BP\) between the control and MP condition was larger in the ventral striatum (-43%, range +19 to -128%) compared to the middle (-27%, range -1 to -46%) and dorsal striatum (-13%, range -5 to -18%). A paired t-test showed a significant difference in \(\Delta BP\) between the control and MP condition in whole striatum \((p = 0.029)\), the dorsal \((p = 0.001)\) and middle striatum \((p = 0.026)\) but not in the ventral striatum \((p = 0.122)\).

**Subjective effects**

MP significantly increased the scores for euphoria \((p = 0.01)\), restlessness \((p = 0.003)\), and desire for MP \((p < 0.001)\). The behavioral response differed between subjects. Most subjects described the experience as pleasurable, only one of the subjects described the effect as partly unpleasurable. Significant correlations were found between change in euphoria and \(\Delta BP\) in the dorsal striatum \((p = 0.006)\) and between baseline anxiety and \(\Delta BP\) in the dorsal \((p = 0.037)\) and middle striatum \((p = 0.005)\). We found a significant negative correlation between baseline \(BP\) in the dorsal striatum and change in euphoria \((p = 0.037)\) (Figure 3).
Figure 2: A: Mean time-activity curves of total RAC uptake for the control and methylphenidate scans. B: Mean (± SD) time-activity curves of the binding ratio \(((C_t - C_{ref})/C_{ref})\) to the occipital cortex for the control scans and methylphenidate scans. CON: control. MP: methylphenidate.
Figure 3: Correlations between % decrease in BP (MP-CON) and change in euphoria (A), % decrease in BP (MP-CON) and baseline anxiety (B) and between baseline BP and change in euphoria (C).
Discussion

In the present study we investigated the effect of a relatively low dose of MP on RAC binding. This is the first study that investigated the effect of MP using a bolus with infusion protocol. The mean RAC BP reached relatively stable values approximately 30 minutes after the start of the infusion. Immediately after MP administration, a clear reduction in striatal RAC binding was seen, suggesting increased DA levels.

Previous human studies have shown that MP or AMPH-induced changes in RAC binding usually ranged between 15-25%, in double bolus or bolus with infusion studies (Breier et al. 1997; Drevets et al. 2001; Martinez et al. 2003; Piccini et al. 2003; Volkow et al. 1994). These studies also reported large differences between the individual subjects. Using a double bolus design, Volkow et al. (1999a) showed that MP (0.25 mg/kg) induced a change in RAC binding that ranged from approximately +5 to -30%. With respect to the method of ligand administration, Carson et al. (1997) reported a larger drug-induced change in RAC binding using a paired bolus design compared to the bolus with infusion method. Although the difference was not significant, they suggest that the methods may differ in sensitivity due to differences in timing of the drug with respect to tracer delivery. However, in another study, the reduction in drug-induced BP did not differ between the double bolus or constant infusion method (Marenco et al. 2004). In our bolus with infusion study, the MP-induced changes in BP were comparable to the changes in the double bolus study by Volkow et al. (1999a).

In our and previous studies, correlations were found between changes in DA release and subjective effects. DA release, especially in the striatum, has been implicated in the processing of rewarding or reinforcing effects of certain stimuli (Ikemoto and Panksepp 1999; McClure et al. 2004; Spanagel and Weiss 1999; Ungless 2004; Wise 2004). Data indicate that DA is released both after unexpected rewards, during expectation of a reward and desire for a reward and also after novel stimuli (Berridge and Robinson 1998; De la Fuente Fernandez et al. 2002; Leyton et al. 2002; Schultz 1998). DA increases have been found both in the dorsal and in the ventral striatum. The DA release in the different subdivisions in the striatum may depend on the nature of the reward and the behavioral response. In our
study, we have found a correlation between the MP-induced change in euphoria and BP change in the dorsal striatum. Previous studies using AMPH have found correlations between drug-induced euphoria and BP changes in the ventral but not the dorsal striatum (Drevets et al. 2001; Martinez et al. 2003). In contrast, Leyton et al. (2002) found that AMPH-induced BP change in the ventral striatum correlated more with “drug wanting” than with mood elevation. Moreover, studies with placebo injections in Parkinson’s patients showed correlations of placebo-induced DA release in the ventral striatum during expectation of the reward (De la Fuente Fernandez et al. 2002) and dorsal striatum during experience of the reward (clinical improvement) (De la Fuente Fernandez et al. 2001). In addition, Small et al. (2003) found that BP change in the dorsal striatum correlated with meal pleasantness and monetary reward-induced DA release has been found in the medial caudate but not in ventral striatum (Zald et al. 2004). Pleasurable experience after smoking was associated with increased DA release in dorsal striatum (Barrett et al. 2004). Previous studies using MP showed that drug-induced euphoria correlated with DA increase in the whole striatum, which according to the authors consisted mainly of the dorsal striatum (Volkow et al. 1999a). The correlation between MP-induced changes in euphoria and BP changes in the dorsal striatum in our study, therefore agrees with the findings from previous studies with MP and studies using non-drug challenges.

In our study, the reduction in RAC was larger in the ventral striatum compared to the dorsal striatum, although not significant. The lack of significance may be due to the high variability in this region. Some previous studies using AMPH have shown larger changes in the ventral striatum (Drevets et al. 2001; Leyton et al. 2002; Martinez et al. 2003) which is in agreement with animal studies that have shown that drugs of abuse preferentially increase DA release in the nucleus accumbens (Di Chiara and Imperato 1988). It is however not known if this also applies to MP.

We found significant correlations between MP-induced BP changes and baseline anxiety. Since MP only blocks reuptake, it is dependent on the extent of physiologically released DA, and therefore on the subjective state of the subject during the experiment (Volkow et al. 1994). Volkow et al.
(1994) has also found correlations between baseline anxiety and MP-induced changes in BP. They postulate that higher anxiety scores may reflect a higher responsiveness of the subjects to novel stimuli and/or unfamiliar situations, which could be linked with a more responsive DA system. In agreement with these findings are studies which show an effect of stress and cortisol on MP- and AMPH-induced increases in DA levels (Marsteller et al. 2002; Oswald et al. 2005).

In addition, in our study a significant negative correlation between baseline BP and change in euphoria was seen. This is also in agreement with studies by Volkow et al. (1999b, 2002b). As Volkow et al. suggest, it is possible that in subjects with a high D₂ receptor density, a smaller dose of MP may have been perceived as pleasant (Volkow et al. 2002b). The low D₂ density could have been caused by high baseline DA levels. However, Volkow et al. (2002b) showed that the measurements were stable over different experiments suggesting that they were not influenced by differences in DA concentration which, in contrast to D₂ density, may fluctuate rapidly between measurements.

There are several methodological limitations that should be considered for this study. Equilibrium was not always achieved in the individual scans, and therefore we used the difference between the MP scans and the control scans for further analyses. Optimally, the control and drug measurements should be made within the same scan. This would need an increase in scan duration in order to attain equilibrium before and after drug administration and requires higher injected activity. In situations of non-equilibrium the estimation of BP may be biased due to effects of ligand distribution or clearance (Carson et al. 1993). In addition, ΔBP may be underestimated if the equilibrium is not established postchallenge (Slifstein et al. 2004). It is currently unknown to what extent these effects may have influenced our results. No MRI scans were made in our study, which would have enabled individual ROI delineation. Unfortunately, this study was not placebo controlled and we did not assess the subjective state during the control condition. Therefore we were unable to distinguish expectation-induced changes in DA from the experience of MP effects. In future studies it is important to control for such variables. In addition, correction for patient
motion will help to increase the sensitivity of the measurements and coregistration with individual MRI scans would enable partial volume correction.

Despite these methodological limitations, our results comply with previous findings, indicating the feasibility of the bolus infusion design combined with a relatively low MP dose to study dopaminergic (dys)function in psychiatric patients.

**Acknowledgments**

We gratefully acknowledge Riemer Slart and Sascha Russo for their contribution to the medical screening of the volunteers and Marijtje van Duijn and Vaclav Fidler for their help with the statistical analysis.
References


receptors in striatal and extrastriatal regions of the primate brain: Single bolus and bolus plus constant infusion studies. Synapse 54: 46-63.


dopamine in brain and of epinephrine in plasma. Psychopharmacology (Berl) 166: 264-270.

